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Sterile Insect Technique

DEVELOPMENT AND STATUS OF THE STERILE INSECT TECHNIQUE FOR MANAGING GYPSY MOTH

R. C. Reardon
V. C. Mastro

Appalachian Integrated Pest Management

USDA Forest Service Northeastern Area
Forest Health Protection

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Development and Status of The Sterile Insect Technique for Managing Gypsy Moth

R.C. Reardon[1]
V.C. Mastro[2]

Introduction

Genetic control has been a part of insect population management for over 35 years. It is a viable management approach for managing pests as it is species-specific and does not produce an impact on the environment. The use of the sterile insect technique for suppression of pest populations was initiated by Knipling (1955) and its effectiveness demonstrated by various models (e.g. Berryman 1967, Knipling 1979). The first successful use of this technique was against the screwworm fly Cochliomyia hominivorax (Coquerel); subsequently, it has been applied operationally and successfully against the Mediterranean fruit fly, Ceratilis capitata (Wiedemann); pink bollworm, Pectinophora gossypiella (Saunders); codling moth, Cydia pomonella (L.); and other species (Whitten and Foster 1975). The potential use of this technology has been documented for numerous additional species (North 1975), although to be operationally usable, the entire continuum of activities associated with deploying the technique (i.e. rearing to field evaluation) needs to be developed (Mastro and Schwalbe 1988).

Most research and operational use of the sterile insect technique has involved the classical approach, in which fertile (wild) populations are overflooded with large numbers of totally sterile individuals. When there is a high overflooding ratio of sterile:wild individuals and the steriles

[1] USDA Forest Service, Northeastern Area, State and Private Forestry, Morgantown, WV 26505
[2] USDA Animal and Plant Health Inspection Service, Otis Methods Development Center, Otis ANGB, MA 02542

are competitive, the probability of mating of females by fertile males declines. This probability decreases dramatically following the release of steriles across consecutive years as the wild population decreases.

Induced inherited sterility is an alternate approach which has been demonstrated in many Lepidoptera species due to their unusual chromosomal structure of diffused centromeres (holokinetic chromosomes). Chromosome translocations are the basis for inherited sterility in Lepidoptera. When a substerilizing dose of radiation is administered to the male parent which is then mated with a non-irradiated female the F_1 progeny are more sterile than the treated parental (P_1) generation and the sex ratio of the F_1 progeny is skewed in favor of males (North 1975, LaChance 1985). These attributes are lacking when irradiated females are mated with normal males. Also, the deleterious effects of radiation on reproductive ability are greater for females than for males (North 1975).

For population suppression, substerilized insects or their F_1 progeny can be released into the population. The advantages of releasing substerilized insects are: (1) they suffer less somatic tissue damage and are thus more competitive than totally sterile insects; (2) the F_1 progeny from substerile and feral crosses are reared in the field and in theory are hardy and in synchrony with the native population; and (3) the suppressive population effect is across at least two life cycles (Knipling 1979, Snow et al. 1972). This latter attribute is critical as all theoretical models comparing inherited sterility with the classical approach have demonstrated that inherited sterility is more effective than release of an equal number of totally sterile insects (LaChance 1985).

The gypsy moth, Lymantria dispar (L), is ideally suited for population management using the sterile insect technique in that it has one generation per year; males may mate several

times; females usually mate only once and produce an egg mass which may contain from 200 to 1600 eggs; and it overwinters as diapausing eggs. This latter characteristic presents an opportunity for producing F_1 sterile egg masses in the laboratory and stockpiling them for subsequent release (Mastro and Schwalbe 1988).

The potential of using the sterile insect technique to manage low-density, isolated, and leading-edge populations of the gypsy moth was recognized early although field releases were relatively unsuccessful until techniques were developed to rear large quantities of quality insects, assess sterile competitiveness and quantify the impact of the release (Mastro et al. 1981). This publication describes the development of the sterile insect technique for managing gypsy moth.

Methods

Laboratory

Mass rearing - It is difficult to economize on mass rearing of the gypsy moth, due in part, to its large size, relatively long developmental period, and susceptibility to the nucleopolyhedrosis virus (Mastro and Schwalbe 1988). The gypsy moth colony being reared at the USDA - Animal and Plant Health Inspection Service facility at Otis Air National Guard (ANG) Base, Massachusetts has been and continues to be the primary source of insects for the sterile insect technique. The New Jersey strain (NJS) has been in continuous production for 40 generations; therefore, it is considered the "standard" colony strain. The quality of the laboratory New Jersey colony is assessed constantly and it is recognized that this colony is different than wild populations in several ways. Some characteristics of this strain are potentially beneficial, for example, its relatively rapid larval development partially offsets delayed development induced

by irradiation of the P_1 parent. Other characteristics can be either beneficial or detrimental, for example, diapause characteristics of the NJS provide for predictable hatch over a short time period; however, this may also provide hatch which is asynchronous with the wild population. A higher incidence of damaged sperm has been found in untreated NJS males; how this impacts the competitiveness of irradiated P_1 males or their F_1 progeny has not been determined but probably is not beneficial. Also, recent studies by Proshold et al (In Press) indicate that F_1 males produced from NJS males transfer sperm less frequently and when sperm is transferred to the females, they have a higher incidence of remating.

Over a number of years, an intermittent rearing problem characterized by neonate mortality, and reduced and arrested larval development has occurred. This deficiency has been named abnormal performance syndrome (APS) and had expressed itself to such a degree that the quality and competitiveness of insects was compromised. Experimental results were compromised because it was unknown prior to release if reared insects or their progeny would display APS. This problem severely impacted all studies involving the NJS including the development of the sterile insect technique and created renewed interest in the need to maintain alternate laboratory production strains. In 1992, results of a multi-agency and - year research effort indicated that APS is, in part, diet related and the nutrition of the parental generation directly affects the performance of their F_1 progeny. Improvements in the rearing system have been made which should assure that the performance of sterilized insects and their F_1 progeny will be more competitive.

Sterility - Efforts were initiated in the late 1950's to determine the doses of gamma irradiation (Cobalt-60) that would produce total sterility in the various life stages of the gypsy moth with minimal somatic tissue damage (Godwin

et al. 1964). Early studies on the use of chemosterilants (e.g. tepa) have not been pursued due to potential hazards to man and other animals. Laboratory studies with sterility continued through the 1960's and focused on the treatment of male pupae (pupae are easier to irradiate than late stage larvae or adults and exhibit the same amount of inherited sterility) with various doses of radiation. These studies were followed by release of totally sterile males. The results of these early efforts were often contradictory due to numerous problems; especially, the variable quality of laboratory reared gypsy moth. In the 1970's, laboratory studies documented that late stage (8-11 day old) male pupae could be completely sterilized with a dose of 15 kilorads (krads) and that the sterile adults were competitive with feral (wild) individuals in flight behavior, pheromone response, mating propensity and other characteristics (Mastro 1980, Waldvogel et al. 1982). In 1980, irradiation studies were initiated to examine the feasibility of using induced inherited sterility. Male pupae were treated with reduced doses of radiation and their subsequent mating with non-irradiated females produced a gradation of sterility in their progeny. The low levels of irradiation induced damage to the genetic material in the sperm. Sterility levels were affected by dose and age of the male pupae treated: the higher the dose the smaller the percent of F_1 eggs which hatch and survive to the adult stage; and the younger the pupae when irradiated the lower the percent hatch, while those aged 7-9 days tend to be most resistant (Mastro et al. 1989). Laboratory studies documented that male moths treated as 8 to 9 day old pupae with 10 krads and mated with non-irradiated females produced egg masses with suppressed hatch (less than 50%), total sterility in the adult stage, and a 2:1 (male:female) sex ratio. The progeny of irradiated females mated with non-irradiated males exhibits some sterility but are usually more fertile than the P_1 female and produce progeny in a normal sex ratio.

In recent laboratory studies, 8 krads (applied to 6-9 day old male pupae) appear to be the optimal dose to induce F_1 sterility with more competitive insects. However, this lower dose provides some fertility in the F_1 generation and may not be acceptable for use in eradication programs.

Field

Host density - The sterile insect technique is best suited for application against low-density populations. To be applied successfully, it is dependent on reasonably accurate characterization of the distribution and density of populations so that adequate numbers of sterile insects can be released to achieve desired overflooding ratios. An accurate estimate of low density gypsy moth populations (less than 50 egg masses (EM) per hectare or 20 per acre) using conventional techniques for counting egg masses is difficult (Wilson and Fontaine 1978), so alternate techniques were developed for monitoring other life stages.

The USDA standard milk carton trap or delta trap with various doses of the (+) enantiomer of disparlure, the synthetic sex pheromone of the female gypsy moth, have proven valuable for describing population distribution and male density (Elkinton and Carde 1980); and to a lesser extent for estimating egg mass density. Additionally, bands (e.g. burlap) wrapped around tree boles have provided artificial niches for estimating larval density, for collecting the various life stages, and for monitoring developmental synchrony, survival and overflooding ratios. Egg masses, placed in screen enclosures to prevent escape, have been used to monitor percent hatch of F_1 eggs and synchrony of their hatch with feral eggs.

There are several methods that can be used to distinguish steriles from ferals: released sterile males can be marked either internally by mixing calco oil red in the larval diet or externally by dusting pupae or adults with a fluorescent

powder. Both methods provide for easy identification of released adults. Also, F_1 identification can be done in the larval stage (4th and 5th instars) by chromosome analysis of males or by field collecting, rearing on artificial diet to the adult stage and back mating with control insects. Normally, one-day-old adult males are mated to virgin laboratory reared females. Resulting egg masses are held 30 days (25°C, 50-60% RH) for embryonation, and mating type determinations are made based on the proportion of eggs which were embryonated and hatched.

The relative mating success of sterile and feral males can be estimated by collecting and evaluating the egg masses from one-day-old, virgin monitor females placed in sheltered locations or from native females located near or under bands, etc. In the former case, resulting egg masses are held for the normal 30-day embryonation period and examined for percentage embryonation and in the latter case, eggs are held for an additional 150 days (4-5°C) to satisfy diapause requirements because the female type is unknown. A determination of the female type is then based on percent hatch of the eggs (Mastro et al. 1989). Holding egg masses produced by females of unknown type (i.e. sterile or feral) is necessary as F_1 females, when mated to normal males, produce highly embryonated egg masses indistinguishable from egg masses produced by normal mating pairs. Nevertheless, these egg masses produced by F_1 females fail to hatch. Field collected egg masses could potentially be the progeny of four possible mating types (Table 1).

Management objective - The use of the sterile insect technique for eradication or suppression of low-density gypsy moth populations requires evaluation of the characteristics of F_1 progeny from the various dose and age treatments. Treating P_1 males with 10 krads in either the 8-9 or 10-11 day age

Table 1. Characteristics of egg masses produced by incrossing and outcrossing F_1 adult gypsy moth progeny of males irradiated (10 krad) as 6-11 day-old pupae mated with normal females.

Type	Mating type (female x male)	n[1]/	Mean proportion of eggs embryonated	SE	Mean proportion of total eggs which hatched	SE
1	F_1 x F_1	50	0.0910	0.0155	0.0012	0.0006
2	Normal x F_1	52	0.2630	0.0380	0.0277	0.0104
3	F_1 x Normal	60	0.6711	0.0353	0.0088	0.0020
4	Normal x Normal	145	0.9374	0.0118	0.7653	0.0154

[1]/ Number of mating pairs producing an egg mass.

groups provides F_1 progeny which are sterile. However, the proportion of F_1 eggs that hatch is only approximately 34-40 percent, mean larval developmental time is approximately three days longer than that of control (laboratory colony) insects and survival is reduced. Only a small number of F_2 larvae (all sterile) result from outcrossing F_1 adults from this treatment group; therefore, when eradication is the objective, this dose and pupal irradiation age may be the best choice. Selection of a lower treatment dose (6 krads) provides F_1 larvae with a faster development time and greater numbers of F_1 adults; these adults, however, are more fertile than F_1 progeny of males receiving higher doses of radiation. The outcrossed F_1 female progeny of 6 krad treated males are also more fertile than the reciprocal crosses. This combination would be a better choice for population suppression (Mastro et al. 1989).

Application techniques - Three different application techniques have been used: (1) deployment of male pupae treated with a sterilizing dose of radiation (15 krads); (2) deployment of male pupae treated with a substerilizing dose of radiation (8 to 10 krads); and (3) broadcasting F_1 sterile eggs from substerilized male and non-irradiated female crosses. Initially, the sterile insect program focused on the strategy of releasing totally or substerilized males as pupae.

Due to high application costs (i.e. pupae must be released daily over the entire male moth flight period) and the limited production time (sterile insects could not be stockpiled), the focus was redirected to the release of F_1 sterile eggs. The eggs can be produced in the laboratory and held in diapause under cold temperatures for months, thereby permitting stockpiling of large numbers. Application costs are reduced as sterile F_1 eggs need to be released only once and the cost of applying eggs can be further reduced by aerial release. The F_1 sterile eggs must hatch in synchrony with feral eggs; and the F_1 sterile larvae must be able to establish, survive and develop in synchrony with the wild population in order to achieve the required overflooding ratio. The F_1 males must be competitive with the ferals to provide the desired reduction in mating success of feral females.

Results

Field tests - During the 1960's and early 1970's, numerous field trials were conducted using totally sterilized males (from pupae treated with several different krad doses). In general, efficacy results were contradictory due to the variable quality of laboratory reared insects, unavailability of reliable techniques for estimating low-density gypsy moth populations and for quantifying the effects of a sterile release.

Intensive evaluation of the totally sterile male technique began in 1977, and culminated in 1980 with a pilot project evaluation in an isolated infestation of gypsy moth (2.59 km^2) in Benton Harbor, Michigan. Releases of sterile pupae (treated with 15 krads as 8-11 day old pupae) were conducted over three years (1980-1982) and the infestation was eradicated (no male moths trapped in 1983-1986). In spite of the technique's effectiveness, there were associated difficulties: male pupae are fragile and special packing/shipping was required, male pupae were released in eclosion cages

(designed to exclude predators and allow for exit of emerged moths) which were costly and difficult to maintain, and since adult males are relatively short-lived (2 or 3 days in the field), frequent releases were necessary to maintain the desired overflooding ratios.

In 1982, in Horry County, South Carolina, a release of substerile pupae (treated with 10 krads as 8-12 day old pupae) was the first pilot test of induced inherited sterility. This release resulted in the occurrence of sterile F_1 individuals and a population decrease in 1983. There was no evidence of a remaining population in 1984, nor in 1985. In 1983, a second evaluation using substerile P_1 male gypsy moths was initiated in Maryland and results showed that sterile F_1 individuals could be found in the field the following year.

These pilot studies with release of totally sterile and substerilized pupae demonstrated efficacy; however, application costs were high and narrow operational and rearing windows remained as obstacles to an operational program.

In 1984, the first pilot project evaluation of sterile F_1 egg masses was conducted in replicated isolated woodlots along the then leading-edge of the gypsy moth infested area in Maryland as part of the Maryland Gypsy Moth Integrated Pest Management Project (Reardon et al. 1987). Even though the development of F_1 larvae was in synchrony with ferals and F_1 survival was adequate, efficacy results were poor as treated populations remained relatively constant or increased as did the controls. In 1985, sterile F_1 egg releases were made against isolated populations in Ohio and Washington to pilot test the technique for eradication of isolated infestations. In 1985, releases of sterile F_1 eggs were made in Vermont and in 1985 and 1986 in Maryland to assess this technique for suppressing low-density building populations within the generally infested area and along the leading-edge, respectively. From 1984 through 1986, approximately

420 ha were treated with F_1 sterile egg masses within the endemic gypsy moth area. Target population density ranged from 2.4 to 91.2 EM/ha. In areas where the gypsy moth had recently invaded (e.g. leading-edge in Maryland Eastern Shore and West Virginia), F_1 sterile egg release did not provide a measurable level of suppression. In areas where the gypsy moth was well established (e.g. Vermont, Pennsylvania, Massachusetts), the rapid increase in host density affected by release of F_1 steriles significantly increased parasitism (especially by Compsilura concinnata) which resulted in native population suppression.

The sterile F_1 egg release in Bellingham, Washington was one of the first efforts against isolated populations and a summary of the results are presented here as it offers the most complete available information on the impact of a sterile F_1 egg release. The infestation was detected in a residential area in 1983 using (+) disparlure baited USDA delta traps (13.9 traps/km^2) and the area was more intensively trapped (58 traps/km^2) in 1984. The infestation was delimited to approximately 40 ha based on the capture of 82 moths and detection of nine egg masses in 1984. Based on the number of moths captured, the target population was estimated to contain a total of 385-400 egg masses. This calculation assumed that, at the density deployed, traps captured 20 percent of the males present, a 1 male: 1 female ratio, and 100 percent female mating success (Schwalbe et al. 1991). Also, it was assumed that 2.5 F_1 egg masses would yield the same number of adult males as one wild egg mass in a single generation (discounting any potential impact of sterile F_1 females). Since the gypsy moth infestation was in a residential area, the F_1 egg masses were released across two years in an effort to minimize the risk of defoliation. In 1985, a total of 34,000 sterile F_1 egg masses was released by hand. This was estimated to be equivalent to 13,600 wild egg masses in terms of the numbers of adult males produced and would achieve a 34:1 overflooding ratio (13,600 F_1 egg

masses: 400 wild egg masses). Egg masses were distributed based on patterns of male moth captures in 1984 and placed at the base of host trees or broadcast in areas of heavy undergrowth. In 1986, an additional 12,769 F_1 sterile egg masses ($\approx$ 5108 wild egg masses) were released mostly in areas where sampling in 1985 detected wild insects. Trapping results in 1987 and 1988 were negative. In spite of successful eradication of the gypsy moth population, several problems were identified: the need for additional techniques for estimating native egg mass densities so that effective overflooding could be achieved; and the interpretation of data from the different monitoring techniques to permit better evaluation of impact of the releases.

Since 1988, eight isolated gypsy moth infestations have been treated using releases of sterile F_1 eggs whose male parents were irradiated (10 krad). In general, the efficacy results were favorable probably, due in part, to excessive overflooding ratios, although numerous problems were identified: 1) how to accurately predict when native egg hatch will occur and how to time the release of F_1 egg masses so that the hatch is synchronous; 2) how to lessen F_1 mortality which occurs mostly in early stage larvae; 3) the impacts of dispersal (neonate and adult) on the overflooding ratios, and 4) the relative competitiveness of immatures.

Recent efforts have focused on synchronizing F_1 egg hatch with wild hatch. The initial approach was to store F_1 eggs at cold temperatures in the laboratory for various lengths of time and just prior (1 to 2 weeks) to anticipated wild hatch to field release them. An alternate approach is to store F_1 eggs on the site in the fall of the year prior to desired treatment. In a number of studies, a wide variety of field egg placement dates of various aged eggs were evaluated and several treatments resulted in synchronous F_1 sterile and wild egg hatch.

The release of substerilized pupae and sterile F_1 eggs was evaluated against low-density populations along the leading-edge of gypsy moth within Rockbridge County, Virginia as part of the 5-year (1988-1992) Appalachian Integrated Pest Management (AIPM) Gypsy Moth Program (Reardon 1991). In general, the efficacy results from the release of substerile pupae were favorable whereas the results for sterile F_1 eggs were not favorable. These techniques were not used operationally as part of the AIPM Gypsy Moth Program due to variable efficacy, high costs, limited production of substerile pupae, and the need for additional data concerning the previously mentioned problems associated with the release of F_1 sterile eggs.

Summary

The sterile insect technique for gypsy moth has involved three different application techniques: (1) deployment of totally sterile male pupae, (2) deployment of male pupae treated with a substerilizing dose of radiation, and (3) broadcasting F_1 sterile eggs. Experience has shown that the deployment of totally sterile male pupae is the least desirable due to the impact being limited to the year of release and the high associated application costs. Releases of substerilized pupae are as labor intensive as releases of totally sterilized pupae; however, the impact on the native population extends over two seasons. Unfortunately, the production of insects for release as pupae is confined to a very narrow time window and therefore, a rearing facility would be under-utilized during the remainder of the year unless alternate projects were in place. The logistics associated with the shipment and field release of pupae are also difficult. Male moths are short-lived (2 to 3 days) and must be released continuously over the normal flight period which is about four weeks. Release of F_1 eggs has the advantages: single release prior to wild egg hatch, a wider production window because diapausing eggs can be stored,

and the logistics of shipment and release are simplified. Therefore, upward adjustments in the release ratio (wild:feral) could be made to overcome errors in estimating basic parameters such as survival rates, competitiveness of released males, etc. Disadvantages of releasing F_1 sterile eggs are: timing of release is difficult, decreased survival of the early stage larvae, and also a potentially damaging stage is released. The probability of detectable defoliation from F_1 releases in native low-density sites, however, is minimal due to the low numbers of insects released. Additional studies are needed to determine the overall quality (fitness) of the NJS of gypsy moth currently used in the APHIS production facility. This includes data on its diapause characteristics and how these affect hatch synchrony with wild populations. Additional data on F_1 immature development and survival; ability of F_1 males to transfer sperm, and tendency to mate less (than wild males) on the same day; and the impact of female multiple mating (approximately 15% of the females mated twice) on viability of resulting egg masses needs to be generated to fully understand and utilize the sterile insect technique.

Conclusion

There has been renewed interest in the use of the sterile insect technique as an operational management tool for low-density gypsy moth populations with the identification of some underlying causes of APS in the NJS colony. All rearing problems, however, have not been fully solved as is evidenced by a recent extended period of low embryonation and hatch in colony eggs. Evaluation methods for the inherited sterility technique are labor intensive and require that the insects be held for a relatively long period of time before a determination of type (F_1 or wild). A method to rapidly discriminate between F_1 and wild insects would allow a more intensive evaluation of releases, and lower the cost of evaluation. Some techniques under investigation which

would provide for a rapid discrimination between wild and F_1 insects have a genetic basis (i.e. larval setal patterns, morphometrics of adult male wing venation) and others, such as paternity assays, may depend on identification of damage induced by radiation (Mastro et al. 1989). The release of F_1 progeny in the egg stage is the preferred application technique (rather than release of sub-sterile or totally sterile male pupae) although the synchronization of F_1 egg hatch with wild egg hatch and reduced survival of early stage larvae remain as major problems for its operational use. The use of this technique to suppress populations should be re-evaluated in isolated low-density populations rather than in similar density populations in the generally-infested area or along the leading-edge as dispersal and other spatial effects place severe limitations on the technique. The effective use of this technique requires the development of additional methods to more accurately estimate population density and spatial distribution, and competitiveness of irradiated insects.

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